Stanley Alan Plotkin's Development of a Rubella Vaccine (1969) [1]

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In the US during the late 1960s, Stanley Alan Plotkin, John D. Farquhar, Michael Katz, and Fritz Buser isolated a strain of the infectious disease rubella and developed a rubella vaccine with a weakened, or attenuated, version of the virus strain. Rubella, also called German measles, is a highly contagious disease caused by the rubella virus that generally causes mild rashes and fever. However, in pregnant women, rubella infections can lead to developmental defects in their fetuses. Plotkin and his collaborators weakened a strain of rubella, called RA 27/3, by growing the virus in WI-38 cells, a strain of human embryonic cells developed at the Wistar Institute by Leonard Hayflick in the early 1960s. Their research led to the development of a rubella vaccine, which prevented rubella in children and congenital rubella syndrome in the fetuses of pregnant women who had contracted rubella.

Plotkin developed vaccines at the Wistar Institute at the University of Pennsylvania [2] in Philadelphia, Pennsylvania, from 1963 to 1969. During that time, Plotkin practiced pediatrics at the nearby Children's Hospital of Philadelphia, in Philadelphia. At the Wistar Institute in 1963, Plotkin began to assemble a team of researchers to isolate a new strain of the rubella virus, RA 27/3, and developing a vaccine for rubella. He enlisted the help of Katz and Farquhar, who both were pediatricians and vaccine researchers at the University of Pennsylvania, to assist in the laboratory research and clinical trials conducted at the Wistar Institute. Plotkin also collaborated with Buser, a pediatrician in Switzerland, to organize and conduct international vaccine trials in later stages of testing of the rubella vaccine. Plotkin's team published their results in 1969.

In 1963, an epidemic of rubella began in Europe, and that epidemic later spread to the US in 1964 and 1965. In the US, there were 12.5 million diagnosed cases of rubella and 20,000 cases of congenital rubella syndrome, which caused infants to be born with abnormal of the eyes, ears, heart, and brain, as well as fetal death and spontaneous abortion [3] of fetuses for pregnant women who had contracted rubella. As a result of those outbreaks, researchers looked to develop a vaccine for rubella to protect against future outbreaks. In 1962 at the Walter Reed Army Institute of Research in Washington D.C., Paul Douglas Parkman and Thomas Huckle Weller isolated the rubella virus for use in developing a vaccine.

Other researchers had attempted to make rubella vaccines, but those vaccines had side effects, such as symptoms of rubella or arthritic joint pain. At that time, most of the attempted rubella vaccines were subcutaneous, meaning that they were injected under the skin. Nasal vaccines for rubella had not worked against the disease, though researchers worked on them because they could be convenient for physicians to administer to children.

Researchers had developed those rubella vaccines using virus strains that they had attenuated, or weakened, by repeatedly growing the virus in animal cells. That process weakened the virus's ability to infect human cells. As the animal cells grew in cultures, the rubella virus adapted to the characteristics of the animal cell lines. Over multiple generations of cells, the rubella virus adapted more to the characteristics of animal cells and less to those of human cells. As a result, the rubella virus became better at targeting and infecting animal cells than it was at targeting and infecting human cells. However, attenuated viruses also caused different or more severe side effects, such as arthritic joint pain.

Plotkin's research team took a different approach to develop a rubella vaccine. They used human cell strains, rather than other animal cell lines, to attenuate the rubella virus for use in a vaccine. Other research teams had used animal cells strains to attenuate strains of the rubella virus because the substantial differences between human and animal cells enabled researchers to weaken the virus easily. However, there was a risk that animal cell lines may carry viruses, in addition to the rubella virus, that might be infectious to humans [4] and that those viruses could contaminate vaccines made using animal cells. Plotkin's team used the human cell strain WI-38 because the cells did not carry the same risk of viral contaminants. Also, WI-38 cells better represented the adult cells of the immune system, having the same number of chromosomes and similar cellular environments.

Plotkin's research team's experiment had three main goals. The first goal was to isolate and weaken the RA 27/3 strain of the rubella virus in human cells so it would be suitable for use in a rubella vaccine. The second goal was to determine whether both injection and nasal vaccines made from the weakened RA 27/3 strain successfully produced immunity to rubella in both children and adults. The third goal was to test whether vaccination with their experimental rubella vaccines caused arthritic symptoms in adult women, as had previous versions of a rubella vaccine. To begin developing a viable [5] rubella vaccine, Plotkin and his colleagues first isolated a new strain of the rubella virus by growing fetal cells infected with the rubella virus. To isolate the RA 27/3 strain, the research team took samples of infected cells from human fetuses with congenital rubella syndrome that had been aborted during the rubella epidemic in 1964. Although other scientists had isolated other strains of the rubella virus, Plotkin's team wanted to obtain their strain directly from infected human tissues. They did so to ensure that they isolated only

viruses that caused rubella or congenital rubella syndrome. The precaution also ensured that the virus did not have any possible contaminants from other laboratories' cell cultures that could affect the development of their vaccine. They used those infected fetal cells to start growing cell cultures and then began passaging them.

Plotkin's team used passaging, also called subcultivation, to grow the rubella virus in cell cultures. Passaging was a process in which the researchers removed a small number of infected cells from an actively dividing collection of cells, called a cell culture, grown in a laboratory. They then implanted the removed cells in a new container with growth medium, nutrients required by the cells, for the cells to continue to divide. A new daughter cell culture resulted that also contained the rubella virus. The team repeated that process again, removing a small number of cells infected with the rubella virus from the daughter cell culture to create yet another new culture. In that manner, the team perpetuated the cell line of the original cell culture in the laboratory and continue to grow the rubella virus.

Through repeated passaging, the process weakened the ability of the rubella virus to infect cells similar to the original cell culture. After many passages, the rubella virus adapted to the different characteristics of newer culture cells and became less specialized for the cells like those from the original culture. When the weakened version of the rubella virus infected cells similar to the original cell culture, the infection was less severe. Because the infection was less severe, infecting viruses did not cause full-strength symptoms of rubella. Yet, the immune system still recognized the rubella virus and produced antibodies specific to the rubella virus to protect against future infections. Furthermore, weakened viruses did not spread as easily between individuals, even in close contact with one another. Researchers could administer vaccines that used a weakened form of the rubella virus without infecting healthy people.

After passaging the fetal cell cultures four times, Plotkin's team extracted the RA 27/3 strain from their cell cultures. They then infected WI-38 cell cultures with the isolated RA 27/3 rubella strain. Leonard Hayflick [6] had derived the WI-38 cell strain from aborted human fetal tissues at the Wistar Institute in the early 1960s. Cells from fetal tissues worked well for virus attenuation and vaccine development because fetal cells easily grew into fibroblasts. Fibroblasts are cells that grew quickly and continuously in laboratory cell cultures, enabling researchers to study them over long periods of time. Plotkin and his research team used WI-38 cells because WI-38 cells grew more easily and reliably in laboratory cultures for longer periods of time than did cells made from fetal cells originally infected with rubella.

Next, Plotkin and his research team passaged the RA 27/3 strain in WI-38 cell cultures eight times at decreasing temperatures to inhibit the ability of the rubella virus to infect individuals at normal human body temperature. The virus weakened after multiple passages, but the team reported being unsure how many passages were sufficient to weaken the rubella virus for use in a vaccine.

At that point, Plotkin's team attempted to create an experimental rubella vaccine from the rubella virus they had weakened through repeated passages. The experimental vaccine consisted of a saline solution containing a small amount of the weakened rubella virus and additional compounds to stabilize and enhance the effectiveness of the vaccine. Plotkin and his colleagues gave subcutaneous inoculations, injections beneath the skin, to several people. Those who received the experimental vaccine experienced less intense symptoms than those from a normal rubella infection. However, they were still contagious and passed the rubella virus to those in close contact with them, so the vaccine did not prevent the spread of the disease. Plotkin's team worked to attenuate the RA 27/3 rubella strain through more passages to make its infections less severe and less likely to spread to other people.

Plotkin and his team continued to passage the RA 27/3 strain through generations of WI-38 cells to weaken the virus further. They tested the strength of the rubella virus by again inoculating several research subjects after a total of eleven, fourteen, fifteen, and seventeen passages in WI-38 cells. The strength of the rubella virus decreased with each successive passage, resulting in fewer and less severe cases of infection. However, the virus was still infectious to people in close contact with those vaccinated. Plotkin and the other researchers continued to passage the rubella virus through generations of WI-38 cells for a total of twenty-five passages. At that point, the repeated passages had weakened the rubella virus so that when they vaccinated individuals with the attenuated RA 27/3 strain, those individuals did not spread the virus to people in close contact with them. The virus, however, was still strong enough to cause an antibody immune response. Vaccinated individuals developed immunity to the full-strength rubella virus and that the attenuated RA 27/3 rubella strain was a good candidate for Plotkin's team to develop into a rubella vaccine.

Plotkin's team began small-scale clinical trials of their rubella vaccine, mostly in children, to determine whether or not the results they observed in their laboratory were replicable in both subcutaneous and nasal vaccines. The researchers selected fifteen families with multiple children ranging from four months to twelve years old and recorded the rubella antibody levels of each family member. They later used that measurement as a baseline to compare individuals' antibody levels after vaccination and exposure to vaccinated individuals. In each of the fifteen families, Plotkin and his team vaccinated a single child subcutaneously with the rubella vaccine. They vaccinated only one child per family to see if the rubella virus spread from vaccinated individuals to healthy individuals and, if so, how extensively.

After several weeks, the researchers again recorded the rubella antibody levels in each of the family members. Plotkin's team discovered that every child they vaccinated had developed much higher rubella antibody levels with minimal negative side effects, but the other family members still had antibody levels similar to their baseline levels. That result confirmed the

laboratory findings of Plotkin's team that the experimental vaccine caused an immune response but did not infect others in close contact to the vaccinated individual.

In late 1967, Plotkin and his colleagues conducted a similar trial of the RA 27/3 rubella nasal vaccine. The researchers selected thirteen children and one adult woman from several families to receive the nasal vaccine. As in the subcutaneous trial, they recorded the rubella antibody levels of each recipient prior to vaccination. After seven weeks, all subjects except one had developed an antibody response to the rubella virus. Moreover, the researchers noted that none of the people in close contact with those that they had vaccinated had an increase in rubella antibodies, further confirming that the rubella vaccine did not make vaccinated people contagious.

Among all of the vaccinated individuals, there were three cases of inflammation in lymph nodes in the neck that researchers attributed to the vaccine. The lymph nodes in the neck were a primary site of infection by the rubella virus, and inflammation was an early symptom of rubella. Almost none of the individuals they vaccinated showed any sign of infection and those that did only experienced minor symptoms, not a full infection.

Furthermore, vaccinated individuals developed antibodies against the rubella virus, meaning that the vaccine exposed individuals to a minimal amount of the virus but still provided immunity against subsequent rubella infections. Previous experimental nasal vaccines with other strains of the rubella virus had not generated enough antibodies to impart immunity. The RA 27/3 strain worked better than other strains as the primary strain for rubella vaccines.

After the success of the subcutaneous and nasal vaccine trials, Plotkin's team conducted larger scale vaccine trials to continue to test the RA 27/3 rubella vaccine's effectiveness. The researchers selected 123 children from 123 families to receive the experimental rubella vaccine, seventy-five subcutaneously and forty-eight nasally. In the 123 families, researchers identified 170 close contacts to the vaccinated children to test whether or not the vaccinated children became contagious with the rubella virus. Before vaccination, Plotkin and his team measured the rubella antibody levels of the children and of the identified close contacts. The team measured the antibody levels again several weeks later. The researchers concluded that rubella antibody levels increased dramatically in nearly all of the vaccinated children, while no one in close contact with them developed an antibody response. Few children had mild adverse reactions to the subcutaneous vaccine. About twelve percent of those nasally vaccinated showed signs of mild lymph node inflammation. The results further indicated that the RA 27/3 strain was a strong candidate for widespread use as both a subcutaneous and nasal vaccine.

Next, Plotkin and his fellow researchers investigated whether or not administering the rubella vaccine, either subcutaneously or nasally, caused arthritic side effects in adult women. Arthritic joint pain was a common symptom of rubella. Because vaccination involved exposing individuals to weakened versions of a virus, some individuals who received rubella vaccines not made from the RA 27/3 strain experienced mild versions of rubella symptoms, such as arthritic joint pain. Arthritic pain as a result of other rubella vaccines was more prevalent in adults receiving the vaccine than in children. Though rubella was more common in children, women needed the rubella vaccine to protect against congenital rubella syndrome during pregnancy [7]. Therefore, Plotkin and his team identified the issue of arthritic side effects in adults as a major concern. The researchers vaccinated twenty-two female student nurses, ten subcutaneously and twelve nasally, with the rubella vaccine. Over the course of two weeks after inoculation, three subcutaneously vaccinated women and two nasally vaccinated women experienced mild side effects. However, none of the women reported any arthritic symptoms.

The research team further tested their finding that the rubella vaccine did not cause arthritic symptoms by expanding their study to 50 nurses in Philadelphia. Plotkin's team found that though some nurses experienced minor side effects like rashes and fevers, none of the nurses experienced arthritic pain. Their findings indicated that the rubella vaccine made from the RA 27/3 strain, unlike other rubella vaccines, did not cause arthritic pain in adult women and was safe for use in pregnant women.

Plotkin's team next tested the RA 27/3 rubella vaccine on an international scale to see if the results that they had seen in small clinical trials held true for larger populations. They worked with medical researchers and health officials across the US, United Kingdom, Switzerland, France, Israel, Iran, Japan, and the Soviet Union. In total, researchers vaccinated 500 people subcutaneously, 392 of whom were children, and 275 people nasally, of which 236 were children. Several weeks later, researchers found that over 99 percent of people vaccinated subcutaneously and over 84 percent of those vaccinated nasally exhibited significantly increased levels of rubella antibodies. They also found that, regardless of vaccination method, no arthritic symptoms occurred nor did any of those vaccinated spread the rubella virus to the people in close contact with them. The results of their international study demonstrated that the RA 27/3 strain rubella vaccine elicited strong rubella antibody responses, through both subcutaneous and nasal inoculation, and protected people from contracting rubella, including children and pregnant women.

At a conference in 1968 to evaluate the best rubella vaccine candidates, Plotkin argued for the use of the RA 27/3 strain over other strains. Many researchers, like vaccine researcher Albert Sabin in the US, speculated that vaccines made from viruses attenuated in human cell strains, like WI-38, might be contaminated by other viruses within the cell strain, specifically viruses that scientists then thought caused cancer. Plotkin argued that human cell lines were safe for use in vaccine development, citing prior research by Leonard Hayflick [6] that demonstrated that human cell strains like WI-38 did not cause cancerous tumor growth and therefore did not contain cancer-causing viruses.

Plotkin's arguments and results led to the use of the RA 27/3 vaccine alongside other rubella vaccines through much of the 1960s and 1970s. In 1971, the RA 27/3 vaccine was incorporated into Maurice Hilleman's measles, mumps, and rubella vaccine (the MMR vaccine). Later, in 1979, the US Centers for Disease Control and Prevention discontinued the use of all other rubella vaccines because the RA 27/3 vaccine produced fewer side effects, particularly with regards to arthritic joint pain. By 2015, 500 million doses of the MMR vaccine containing the vaccine developed by Plotkin's team had been administered worldwide.

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